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(54) Use of MHC class II ligands (CD4 and LAG-3) as adjuvant for vaccination and of LAG-3 in cancer treatment

(57) The present invention concerns a use of a MHC class II ligand, such as CD4 and LAG-3, for the manufacture of a medicament for preventing or treating pathological conditions involving an antigen specific immune response, as well as the use of LAG-3 in cancer-immunotherapy.

The invention also concerns a pharmaceutical composition comprising an effective amount of an antigen able to induce an antigen-specific immune response together with an effective amount of a MHC class ligand wherein said MHC class II ligand is present as an adjuvant-like agent.

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Description

The present invention relates to the use of LAG-3 and CD4, and in a more general way, the use of MHC class II ligands as adjuvants for vaccines, in order to boost an antigen specific immune response, as well as the use of LAG-3 as a therapeutical agent in cancer immunotherapy.

It is now recognized that the proteins encoded by MHC Class II region are involved in many aspects of immune recognition, including the interaction between different lymphoid cells such as lymphocytes and antigen presenting cells. Different observations have also shown that other mechanisms which do not take place via CD4 participate in the effector function of T helper lymphocytes.

The lymphocyte activation gene 3 (LAG-3) expressed in human $CD4^+$ and $CD8^+$ activated T-cells as well as in activated NK cells encodes a 503 aminoacids (aa) type I membrane protein with four extracellular immunoglobulin superfamily (IgSF) domains (1) and is a ligand for MHC class II molecules (2). Analysis of this sequence revealed notable patches of identity with stretches of aminoacids sequences found at the corresponding positions in CD4, although the overall aminoacids sequence homology with human CD4 is barely above background level (approximately 20 % sequence identity). There are also some internal sequence homologies in the LAG-3 molecule between domains 1 (D1) and 3 (D3) as well as between domains 2 (D2) and 4 (D4) suggesting that LAG-3 has evolved like CD4 by gene duplication from a preexisting 2 IgSF structure (1). In addition, LAG-3 and CD4 genes are located in a very close proximity on the distal part of the short arm of chromosome 12 (3). LAG-3 and CD4 can therefore be regarded as evolutionary "first cousins" within the IgSF (2).

Like CD4, hLAG-3 is composed of Ig like ectodomains with a WxC signature motif in domains 2 and 4; however a difference with CD4 is the presence of an extraloop sequence in domain 1 (recognized by the 17B4mAb) and an intracytoplasmic proline rich motif (EP repeats) in human LAG-3 (hLAG-3). Recently, murine lymphocyte activation gene 3 (mLAG-3) was cloned and approximately 70 % of homology was found with hLAG-3, with the same proline rich motif in the intracytoplasmic tail.

Antigen specific stimulation of $CD4^+$ T-cell clones in the presence of anti-LAG-3 mAb leads to extended proliferation and cytokines production (5). It has been suggested a regulatory role of hLAG-3 on $CD4^+$ T lymphocyte activated, by cross-linking MHC class II molecules expressed on T-cells with LAG-3 Ig fusion proteins (6). LAG-3 MHC class II interaction inhibits signals through MHC class II molecules expressed on $CD4^+$ T-cells (decrease of proliferation and cytokines production), suggesting that both LAG-3 and MHC class II are effector molecules for the down-regulation of T helper cell mediated immune responses. The hLAG-3 Ig fusion protein was found to bind xenogenic MHC class II molecules (murine and monkey). In addition, the mLAG-3 has been proposed to transduce a positive signal in effector cells, since transgenic mice with a LAG-3 null mutation have a defect in the NK cell compartment (7).

Mouse tumor cell lines engineered to express membrane (B7.1, B7.2, CD95L, ...) or secreted molecules (IL-2, IL-12 ....) are often used to investigate immune responses or antitumor effects. This approach implies that many tumor cells are potentially antigenic (9), and become immunogenic when they express molecules. Experimental mouse tumors are classified as intrinsically immunogenic when, after a single injection into syngenic mice as nonreplicating cell vaccines, they elicit a protective immune response against a subsequent lethal challenge. Tumors that do not retain this residual immunogenicity are defined as poorly immunogenic or nonimmunogenic. The inventors of the instant application have investigated whether hLAG-3, human CD4 (hCD4) and mLAG-3 expression on two MHC class II-mouse tumors (the poorly immunogenic sarcoma MCA 205 and the nonimmunogenic TS/A adenocarcinoma) can mediate an immune response so as to reject mouse tumor and can induce systemic immunity.

The authors of the present invention have discovered that human or murine LAG-3, whether expressed as membrane proteins in solid tumor cell lines or inoculated into mice as a soluble protein induced a potent immunity against highly malignant murine tumors. The immunity was T-cell dependent and antigen-specific.

They have further investigated the role of CD4 and found that human CD4 (hCD4) also induced a systemic antitumor response.

The induced immunity has been found to be T-cell mediated since the same antitumor response was obtained with Nude mice lacking T-lymphocytes.

The antitumor effect was still found when using different tumor cell lines exhibiting different intrinsic immunogenicity as well as different strains of mice expressing different MHC genes.

Furthermore, the hLAG-3 and hCD4 induced effects were observed when tumor cell lines expressing hLAG-3 or hCD4 were injected at a distant site from the initial inoculation site of the wild-type tumor cell lines.

Furthermore, systemic administration of soluble hLAG-3 directly induces an inhibition of *in vivo* tumor growth.

All the aforementioned results demonstrate that LAG-3 and CD4 are able to elicit an antigen specific T-cell mediated immune response and may be useful as a tool in immunotherapy, in order to prevent the occurrence of a cancer among populations at risk or more generally in any immunotherapy involving an antigen-specific T-cell mediated immune response, and that LAG-3 is further useful as a tool for inhibiting *in vivo* tumor growth.

According to one of its aspects, the present inven-

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tion relates to the use of a MHC class II ligand for the manufacture of a medicament for preventing or treating pathological conditions involving an antigen specific immune response, preferably an antigen-specific T-cell mediated immune response.

In a first embodiment, the MHC class II binding molecule is LAG-3 as well as derivatives thereof, able to bind the MHC class ligand of LAG-3.

By derivatives of LAG-3, in the sense of the present invention, there are meant mutants, variants and fragments of LAG-3 namely soluble fragments of LAG-3 provided that they maintain the ability of LAG-3 to bind MHC class II molecules.

Thus, the following forms of LAG-3 may be used:

- the whole LAG-3 protein,
- a soluble polypeptide fragment thereof consisting of at least one of the four immunoglobulin extracellular domains, namely the soluble part of LAG-3 comprised of the extracellular region stretching from the aminoacid 23 to the aminoacid 448 of the LAG-3 sequence disclosed in French Patent application FR 90 00 126,
- a fragment of LAG-3 consisting of substantially all of the first and second domains
- a fragment of LAG-3 consisting of substantially all of the first and second domains or all of the four domains, such as defined in WO 95/30750, such as
- a mutant form of soluble LAG-3 or a fragment thereof comprising the D1 and D2 extracellular domains and consisting of:
- a substitution of an aminoacid at one of the following positions:

  - position 73 where ARG is substituted with GLU,
  - position 75 where ARG is substituted with ALA or GLU,
  - position 76 where ARG is substituted with GLU,

  or a combination of two or more of those substitutions,
- a substitution of an aminoacid at one of the following positions:

  - position 30 where ASP is substituted with ALA;
  - position 56 where HIS is substituted with ALA;
  - position 77 where TYR is substituted with PHE;
  - position 88 where ARG is substituted with ALA;
  - position 103 where ARG is substituted with ALA;
  - position 109 where ASP is substituted with GLU;
  - position 115 where ARG is substituted with ALA;

  or a deletion of the region comprised between the position 54 and the position 66,

  or a combination of two or more of those substitutions.

Those mutants are described in PNAS, June 1997(4)

- or a physiological variant of LAG-3 comprised of a soluble 52 kD protein containing D1, D2 and D3.

According to a second embodiment, the MHC class II binding protein is CD4 or a derivative thereof able to bind the MHC class II ligand of CD4.

The derivatives of CD4 are such as defined for the derivatives of LAG-3. They are namely mutants, variants and fragments of CD4 namely soluble fragments of CD4 provided that they maintain the ability of CD4 to bind MHC class II molecules.

LAG-3 and CD4, namely hLAG-3 and hCD4 or the derivatives thereof such as defined above may be administered as recombinant moieties expressing said molecules, for example transfected cells or recombinant viruses.

The present invention relates also to tumor cells transfected with a DNA coding for at least one MHC class II ligand, such as CD4 or LAG-3 or derivatives thereof.

A further object of the instant invention is also the use of cells, like tumor cells, transfected with a DNA coding for at least one MHC class II ligand, such as CD4 or LAG-3 or derivatives thereof for the manufacture of a medicament, preferably a medicament for preventing or treating pathological conditions involving an antigen specific immune response like an antigen specific T-cell mediated immune response or for treating pathological disorder like cancers.

The transfected cells are preferably mammal cells and in particular mammal tumor cells.

According to one of its aspects, the present invention relates to a process for preparing cells transfected with a DNA coding for at least one MHC class II ligand, such as CD4 or LAG-3 or derivatives thereof comprising the steps consisting of removing cells from a patient, transfecting said cells with a DNA coding for at least one MHC class II ligand, such as CD4 or LAG-3 or derivatives thereof and recovering the so-transfected cells.

For the preparation of tumor cells according to the invention, this process will be reproduced on tumor cells removed from a patient.

However, according to a preferred embodiment, the MHC class II binding protein, namely CD4 or LAG-3 or the derivative thereof, is administered in a free form, namely in a soluble form by inoculating them systemically, for example as an s.c, i.m or i.v injection.

The medicament according to the invention may be used as a vaccine to prevent disorders associated with an antigen specific immune response, preferably a T-cell mediated immune response.

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To that end, it is administered in a suitable vehicle together with one or several antigen(s) against which an immune response is sought. The antigen may be an inactivated or attenuated infectious agent or a purified antigen, eventually obtained by protein recombinant procedures, such as an antigen of an infectious agent or a tumor antigen, which preferably are able to elicit a T-cell mediated immune response.

The vaccine may be used to prevent a subject against an infectious disease, such as a viral or bacterial disease wherein the infectious agent elicits an antigen specific immune response, preferably a T-cell mediated immune response.

The vaccine may also be used to prevent a subject against cancer, either solid tumor cancer or leukemia.

In that case, the MHC class II binding protein namely LAG-3 or CD4 is administered to a subject either subcutaneously, intradermically or as a nasal spray together with one or several antigens able to elicit an immune response, preferably a T-cell mediated immune response. The antigen may be a peptide, a lipopeptide, a recombinant protein or DNA coding for these antigens.

The anti-cancer vaccine may be inoculated to populations at risks identified by their genotype (preventive vaccine) or to patients (therapeutic vaccine) bearing a tumor or at high risk of relapse following surgery.

A further object of the instant invention is thus a pharmaceutical composition comprising an effective amount of a MHC class II ligand in combination with an effective amount of an antigen able to stimulate the immune system, preferably via a T-cell response.

In still another aspect, the present invention relates to the use of LAG-3 as a medicament for anti-cancer immunotherapy in patients bearing a cancerous tumor.

In that case, LAG-3 is administered preferably as a free LAG-3 protein or a derivative thereof in a pharmaceutically acceptable vehicle preferably a soluble derivative such as defined previously.

LAG-3 may be administered as an intratumoral injection or systemic injection, for example s.c, i.v or i.m.

A further object of the present invention relates to a method for tumor gene therapy comprising the steps consisting of removing a portion of a patient tumor cells, transfecting said cells with a DNA coding for at least one MHC class II ligand, such as CD4 or LAG-3 or derivatives thereof and reintroducing the so-transfected cells into the patient.

The following examples demonstrate the activity of LAG-3 and CD4 in the prevention or treatment of pathological conditions involving a T-cell mediated immune response.

For the better understanding of the invention, it may be referred to the annexed figures wherein:

- figure 1 represents the mean tumor size of C57BL/6 mice inoculated with wild-type MCA 205 tumor cells (MCA WT), MCA 205 tumor cells transfected with hCD4 (MCA hCD4) and MCA 205 tumor cells transfected with hLAG-3 (MCA hLAG-3);
- figure 2 represents the results (mean tumor size) obtained after rechallenging the same mice with wild-type MCA tumors cells at a minimal tumorigenic dose;
- figure 3 represents the results (mean tumor size) obtained after rechallenging the same mice with the irrelevant MC 38 tumor cell line;
- figure 4 represents the results (mean tumor size) obtained with a different strain of mice (BALB/c) and a different tumor cell line (TS/A) either of wild type (TS/A wt) or transfected with hCD4 (TS/A hCD4) or hLAG-3 (TS/A hLAG-3);
- figure 5 represents the results (mean tumor size) obtained with existing tumors treated with different doses of MCA cells expressing hLAG-3;
- figure 6 represents the results (mean tumor size) obtained with soluble LAG-3 injected together with MCA cells (MCA wt, MCA wt + 25 µg LAG-3 and MCA wt + 250 µg LAG-3);
  the figure inside the frame of figure 6 represents the percentage of mice with tumor.
- figures 7 and 8 represent results of expression of LAG-3 in the membrane of tumor infiltrating lymphocytes (TILs) in five patients (P1 -P5) bearing a renal cell carcinoma (RCC).

The experiments illustrated in the examples were carried out by using the following materials and methods.

MATERIALS AND METHODS

1 Tumor cell lines

The tumor cell lines used were : the methylcholanthrene-induced sarcoma MCA 205 cell line, (syngenic from C57BL/6 H-2^b mice) and one cell line syngenic from BALB/c (H-2^d) : the spontaneous undifferentiated mammary adeno-carcinoma TS/A cell line. The MC38 colon carcinoma cell line (syngenic from C57BL/6 mice) was used in rechallenge experiment as control tumor. Cells were maintained at 37°C in a humidified 10 % CO_2 atmosphere in air, in complete medium (RPMI 1640 culture medium supplemented with glutamine, sodium pyruvate, penicillin/streptomycin, 10 % of endotoxin free fetal calf serum and 0.05 mM 2-β mercapto-ethanol). For immunostaining experiments and *in vivo* experiments, cells were removed from their culture vessels with PBS containing 1 mM EDTA. Before sub-cutaneous injection (s.c), cells were washed three times in cold PBS 1X and resuspended in same buffer. Cells were not cultured for longer than two weeks.

2. Genetic constructs

The cDNAs of hLAG-3, mLAG-3 and hCD4 were

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cloned into NT hygromycin plasmid vector, under SRα promoter (10). All tumor cell lines (2.5 x 10^6 cells) were transfected by electroporating using an Eurogentec apparatus (Belgium) : MCA 205 cells at 200V or TS/A cells at 300V, 1500 µF. Transfectants were selected in hygromycin B (Sigma) : MCA 205 in 100 µg/ml and TS/A transfectants in 200 µg/ml. Resistant cells expressing the transfected molecules were identified using a Elite cytofluorimeter (Coulter, Hialeah, FL) and cloned by limiting dilution. The best clone for each construction in each tumor cell lines was used in this study.

3. Cytofluorimetric analysis

Resistant cells expressing the transfected molecules were stained by indirect immunofluorescence, with saturating amounts of purified or ascites fluids mAbs. Cells were first incubated with mAbs : 17B4 (anti-hLAG-3.1) (2), OKT4 (anti-hCD4), a rabbit preimmunserum (termed PIS) used as negative control and a rabbit immunserum anti-mLAG-3 (termed IS). The expression of murine MHC class I and II molecules on tumors were detected with the following mAbs: 34-1-2S for H-2 K^d and D^d, 28-8-6S for H-2 K^b and D^b , 14-4-4S (for E^d), M50114 (for IA and IE).

Cells were then washed and incubated with FITC-conjugated goat anti-mouse serum (GAM Coulter) or FITC-conjugated goat anti-rabbit serum (GAR Southern Biotechnologies Inc.). To study presence of infiltrating cells or recruitement of cells in tumor periphery, some mice were killed and tumor dissociated. Cells were stained by direct immunofluorescence, with 17B4-FITC or the following mAbs (Pharmingen) : anti-mCD4-PE (L3T4), anti-mCD8 (Ly-2 and Ly-3.2), anti-mNK (2B4) and anti-mCD22 (Lyb-8.2). Cells were sorted using a Elite cytofluorimeter Coulter).

4. Mice

Female C57BL/6 mice, 6 or 8 weeks old, were purchased from IFFRA-CREDO Laboratories (Lyon, France). Female BALB/c mice, 4 to 8 weeks old were purchased from JANVIER Laboratoires, (France). All this mice strains were raised in specific pathogen free conditions. Female Nude were purchased from the animal facility of Institut Gustave Roussy and kept under protected microenvironments.

5. *In vivo* tumor experiments

5.1 Tumor establishment and vaccination

Establishment of tumor cell lines was performed s.c using the minimal tumorigenic dose (MTD) at 2.10^5 cells/mouse for MCA 205 and 5.10^4 cells for TS/A or fivefold the MTD. Mice tumor free 30 days after injection were rechallenged with parental tumor cell line (MTD or5 x MTD). MC38 colon carcinoma cell line was used at 10^5 cells as a control tumor in C57BL/6 mice that rejected TS/A tumor. Age-matched naive C57BL/6 or BALB/c mice were injected with tumor cell lines.

Tumor growth was monitored two to three times a week by measuring two perpendicular tumor diameters using calipers. At day *in vivo* tumor experiments, cells were analysed by cytofluorimeter and *in vitro* proliferation assay made.

5.2 Tumor therapy models

On day 0, wild-type tumor cell lines were inoculated s.c. in the left flank (MTD). On day 0, 3 or 6, LAG-3^+ tumor cells were injected into the right flank (MTD or fivefold MTD) to determine the antitumor effects on the nontransfected cells at a distant site. Tumor growth was monitored as described before.

RESULTS

EXAMPLE I:

Surface expression of hCD4, hLAG-3, mLAG-3 and MHC molecules on tumor cell lines.

Transfected tumor clones were stained as detailed in section 2.2, and analyzed to compare level expression of hCD4, hLAG-3 and mLAG-3. The best clone for each construct was used in this study. The following tumor cell lines TS/A express high levels of MHC class I molecules and MCA 205 express low levels of MHC class I. No significant difference was observed between MHC class I expression on parental tumor cell lines compared to transfected clones.

EXAMPLE II:

Tumor establishment models and vaccination : Comparative effects of hCD4 and hLAG-3.

These experiments were performed to examine the tumorigenicity of cells after gene transfection (MCA 205 and TS/A) as shown in figures 1 and 4. The induction of antitumor immunity of LAG-3^+ tumor was compared to parental tumor cell lines.

Animals receiving MCA-LAG-3 rejected their tumor. Animals receiving MCA-CD4 exhibited a lower tumor growth than animals receiving wt MCA. Two of them (over 5) completely rejected the tumor (figure 1).

Similar results were obtained with mLAG-3 (data not shown).

These results indicate that ectopic expression of hLAG-3, mLAG-3 and hCD4 increases the immunogenicity of MCA sarcoma cell line and prevents tumor formation of MCA transfectant, i.e. it induces a potent immunity against a highly malignant murine tumor.

Similar results are obtained with TS/A tumor cells in BALB/c mice (figure 2).

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Thus, antitumor effect is obtained:

- in different strains of mice expressing different MHC complex genes ;
- using different tumor cell lines (exhibiting different intrinsic immunogenicity, TS/A < MCA).

Nude mice were inoculated with wt MCA, MCA hLAG-3 and MCA hCD4 type tumor cells and transfectants grew similarly.

This substantiates the fact that systemic-, long-lasting-, tumor specific-hLAG-3 or hCD4- boosted immunity are T cell mediated.

Mice previously inoculated with wt MCA, MCA hLAG-3 and MCA hCD4 and tumor free after 30 days injection with MTD were rechallenged (1 fold) with five-fold the MTD parental tumor cell line or with an unrelated syngenic MC 38 colon carcinoma cell line.

The results are represented in figures 2 and 3.

After rechallenge, growth of wt MCA was delayed in surviving animals both for animals receiving MCA-LAG-3 cells and MCA-CD4 cells (figure 2).

No such effect was observed in animals rechallenged with irrelevant tumor MC38 (figure 3).

This indicates that both ectopic expression of hLAG-3 and hCD4 have an adjuvant-like effect and induces a long-lasting antigen specific immunity against the unmodified parental tumor.

EXAMPLE III:

Therapy of wt MCA 205 tumors in C57BL/6 mice with MCA-hLAG-3

Three groups of five mice each were used for the experiment.

Each group was inoculated in one flank with wt MCA and three days later, with either MCA wt (group 1), MCA-hLAG-3 at 2.10^5 cells (group 2) and MCA-hLAG-3 at 1.10^6 cells (group 3).

The size of the original tumor was measured in each group over 30 days. The results are represented in figure 5.

Injection of MCA-hLAG-3 delayed the tumor growth in a dose dependent manner.

This experiment confirms the systemic effect of LAG-3 on tumor growth and indicate that LAG-3 represents a therapeutic agent against solid tumors.

EXAMPLE IV:

Therapy of wt MCA 205 tumors in C57BL/6 mice with soluble LAG-3.

Three groups of five mice each were simultaneously inoculated with either wt MCA suspended in either PBS (group 1) or PBS containing soluble human LAG-3 (shLAG-3 D1 D4) in amounts of 25 µg (group 2) or 250 µg (group 3).

The size of the tumors were measured for each group over a period of 30 days.

The results are represented in figure 6.

Co-administration of hLAG-3 D1 D4 induced a dose dependent tumor growth retardation.

This demonstrates that systemic administration of soluble hLAG-3 directly induces an inhibition of in vivo tumor growth.

EXAMPLE V:

***In vivo* LAG-3 expression on human tumor lymphocytes infiltrating (TILs) renal cell carcinoma (RCC).**

Five patients were investigated for LAG-3 expression on tumor infiltrating lymphocytes in renal cell carcinoma tumors.

Dissociated tumors were used for experiments by direct immunofluorescence. The expression of LAG-3 was studied among the lymphocyte population, determined by its size and granulosity. Dead cells were excluded from the study by staining with propidium iodide. TILs were positively stained with 17B4, a monoclonal antibody specific of the epitope of the extraloop of LAG-3.

The results are represented in figure 7 for patients P1-P3 and figure 8 for patients P4 and P5.

For all patients, a shift of the fluorescent peak was observed showing the binding of the 17B4 antibody at the surface of the lymphocytes.

Thus, in all patients, TILs actually expressed LAG-3 with a significant percentage (30 %) of RCC-TIL in unrelated patients.

In contrast, peripheral blood mononuclear cells were LAG-3$^-$ in these patients, showing that LAG-3 expression on lymphocytes is a phenomenon related to T-cell activation in tumors.

Furthermore by using an ELISA assay, high concentrations (about 1 ng/ml) of soluble LAG-3 were found in blood of patients with cancer.

These data show that LAG-3 is a molecule involved in the naturally occurring antitumoral response in human and support the use of LAG-3 to boost the immunosurveillance of tumor cells in human.

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Claims

1. The use of a MHC class II ligand, such as CD4 or LAG-3 for the manufacture of a medicament for preventing or treating pathological conditions involving an antigen specific immune response.

2. The use of a MHC class II ligand, such as CD4 or LAG-3 for the manufacture of a medicament for preventing or treating pathological conditions involving an antigen specific T-cell mediated immune response.

3. The use according to claim 1, wherein the MHC Class II binding membrane molecule is CD4 or LAG-3 as well as derivatives thereof able to bind the corresponding Class II MHC ligand.

4. The use according to claim 1, wherein the MHC Class II binding membrane molecule is LAG-3, mutants thereof or a soluble fragment thereof.

5. The use according to claim 4, wherein the LAG-3 soluble fragments are selected from the group consisting of D_1-D_2 and D_1-D_4 fragments of LAG-3.

6. The use according to claim 1, wherein the medicament comprises the said MHC class II ligand in the form of transfected cells expressing said ligand or in the form of a soluble molecule of that ligand.

7. The use according to claim 1 or 2, for the manufacture of a vaccine for preventing the occurrence of a pathological disorder or for treating the disorder once it has occurred.

8. The use according to claim 1 or 2, for the manufacture of a vaccine for preventing the occurrence of a pathological disorder, which course is influenced by an antigen-specific T-cell mediated immune response, or for treating the disorder once it has occurred.

9. The use according to claim 1, wherein the pathological disorder is a cancer.

10. The use of LAG-3, for the manufacture of a medicament for anti-cancer immunotherapy.

11. A pharmaceutical composition comprising an effective amount of an antigen able to induce an antigen-specific immune response together with an effective amount of a MHC class ligand wherein said MHC class II ligand is present as an adjuvant-like agent.

12. A pharmaceutical composition comprising an effective amount of an antigen able to induce an antigen-specific T-cell mediated immune response together with an effective amount of a MHC class II ligand wherein said MHC class II ligand is present as an adjuvant-like agent.

13. A pharmaceutical composition according to claim 12, wherein said MHC class II ligand is hLAG-3 or hCD4.

14. A pharmaceutical composition according to claim 12, wherein said MHC class II ligand is human soluble LAG-3.

15. A pharmaceutical composition according to any one of claims 12 to 14 as a vaccine.

16. Tumor cells transfected with a DNA coding for at least one MHC class II ligand, such as CD4 or LAG-3 or derivatives thereof.

17. The use of cells transfected with a DNA coding for at least one MHC class II ligand, such as CD4 or LAG-3 or derivatives thereof or of tumor cells according to claim 16 for the manufacture of a medicament as defined in claims 1, 2 and 7 to 10.

18. A process for preparing cells transfected with a DNA coding for at least one MHC class II ligand, such as CD4 or LAG-3 or derivatives thereof comprising the steps consisting of removing cells from a patient, transfecting said cells with at least one MHC class II ligand, such as CD4 or LAG-3 or derivatives thereof and recovering the so-transfected cells.

19. A process for preparing tumor cells transfected with a DNA coding for at least one MHC class II ligand, such as CD4 or LAG-3 or derivatives thereof comprising the steps consisting of removing tumor cells from a patient, transfecting said tumor cells with at least one MHC class II ligand, such as CD4 or LAG-

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3 or derivatives thereof and recovering the so-transfected tumor cells.

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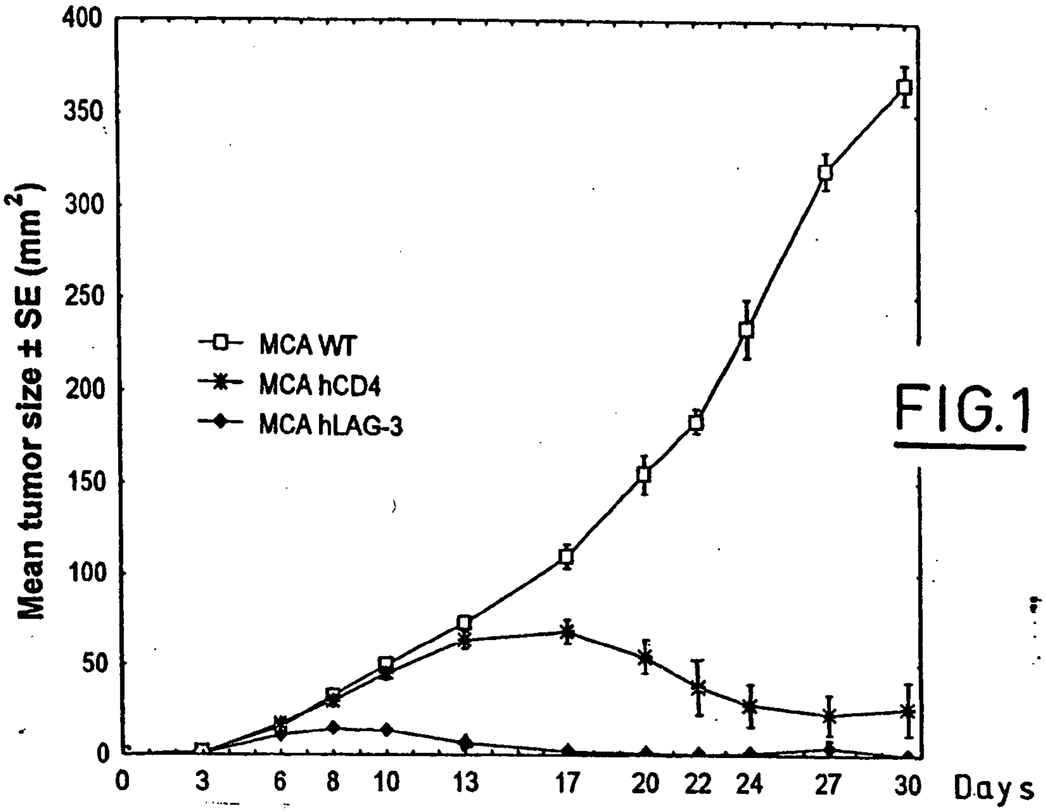

FIG.1

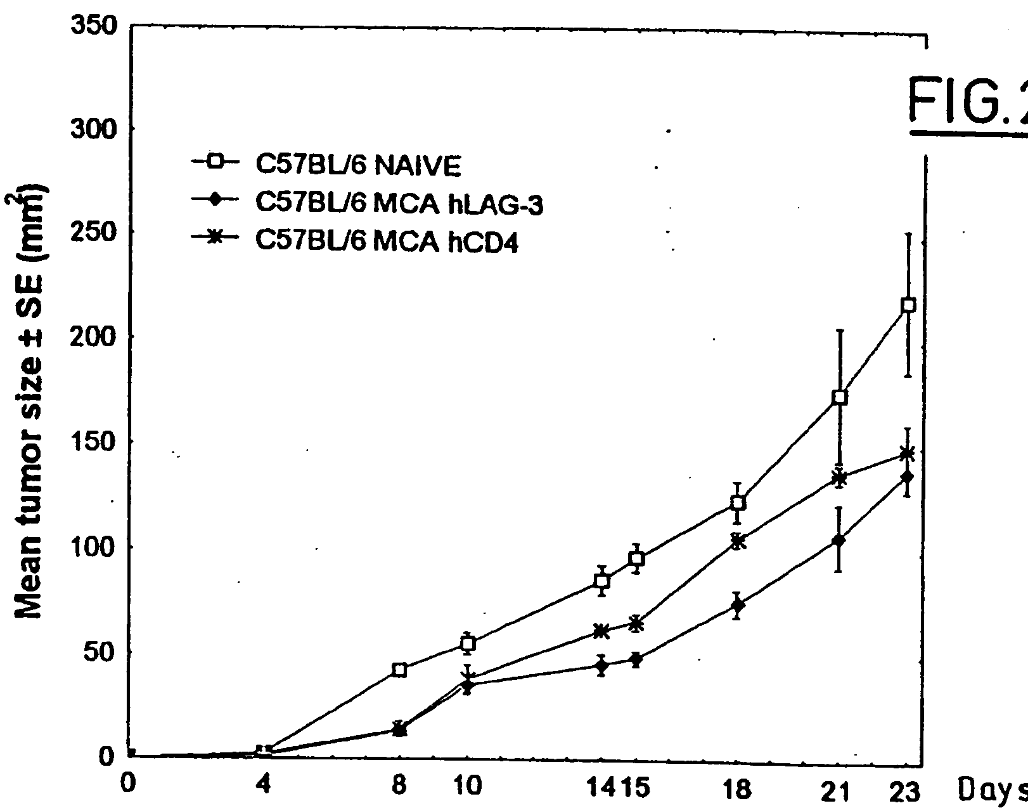

FIG.2

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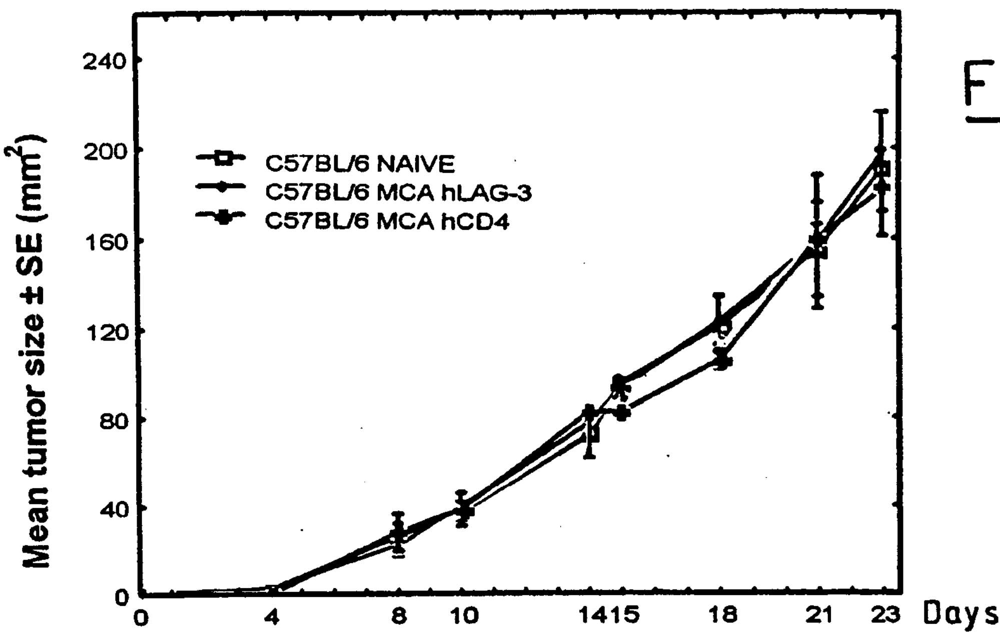

FIG.3

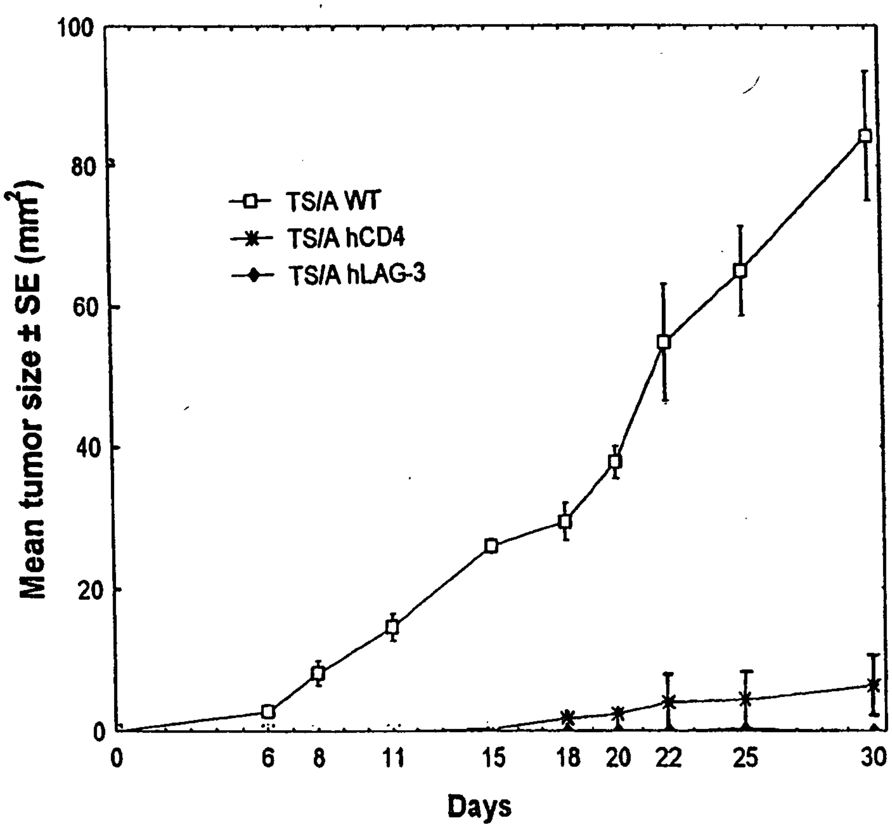

FIG.4

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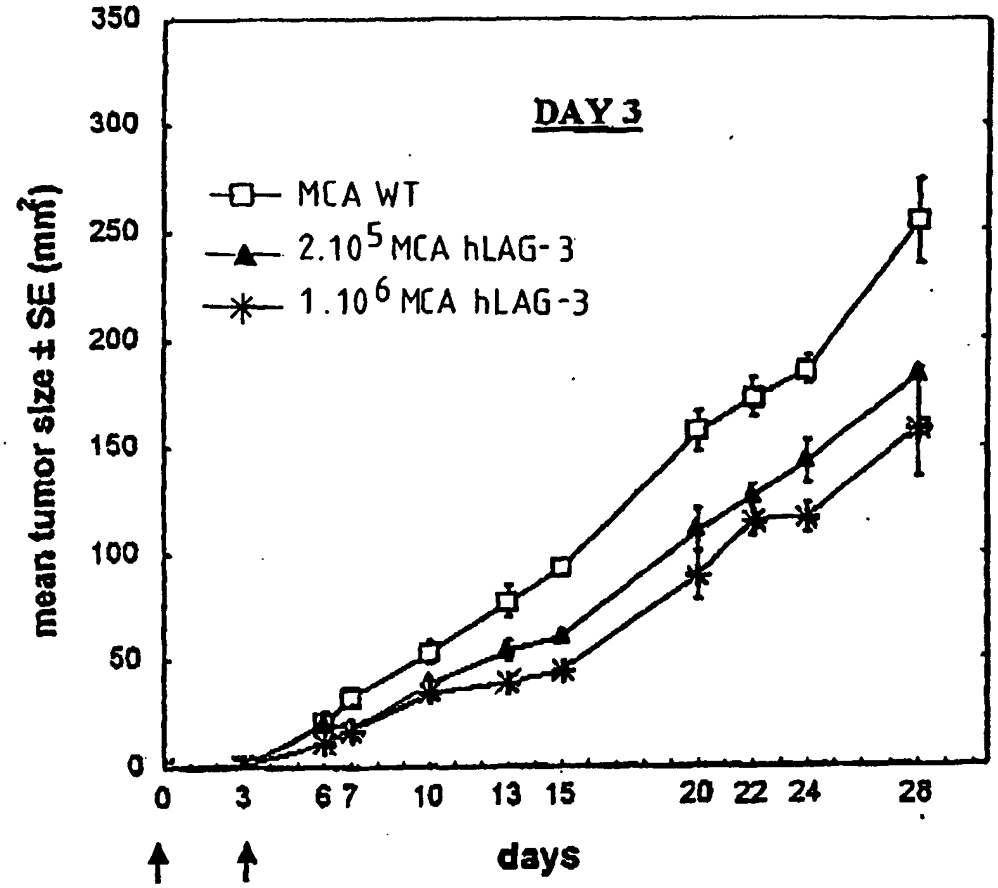

FIG.5

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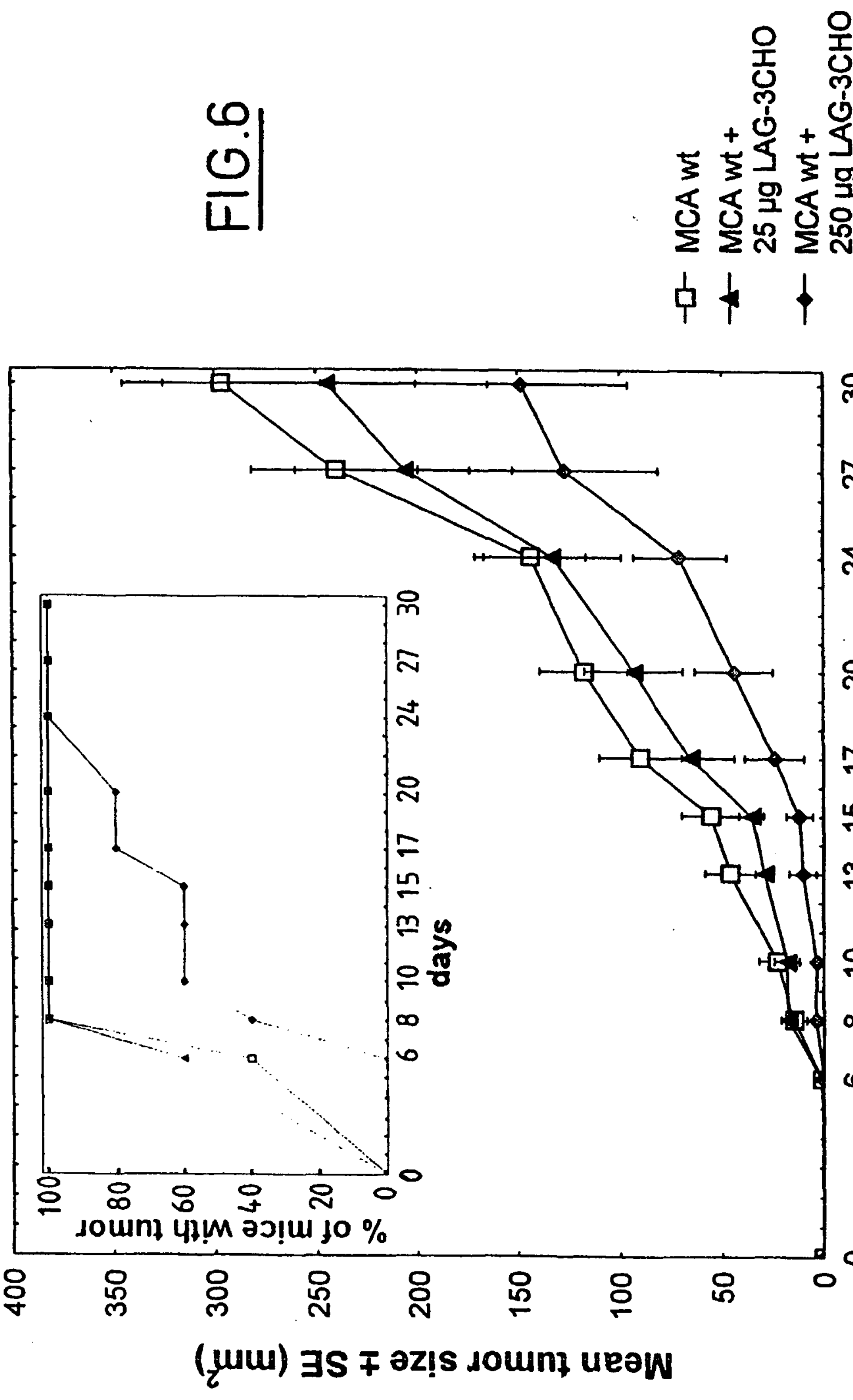
FIG.6



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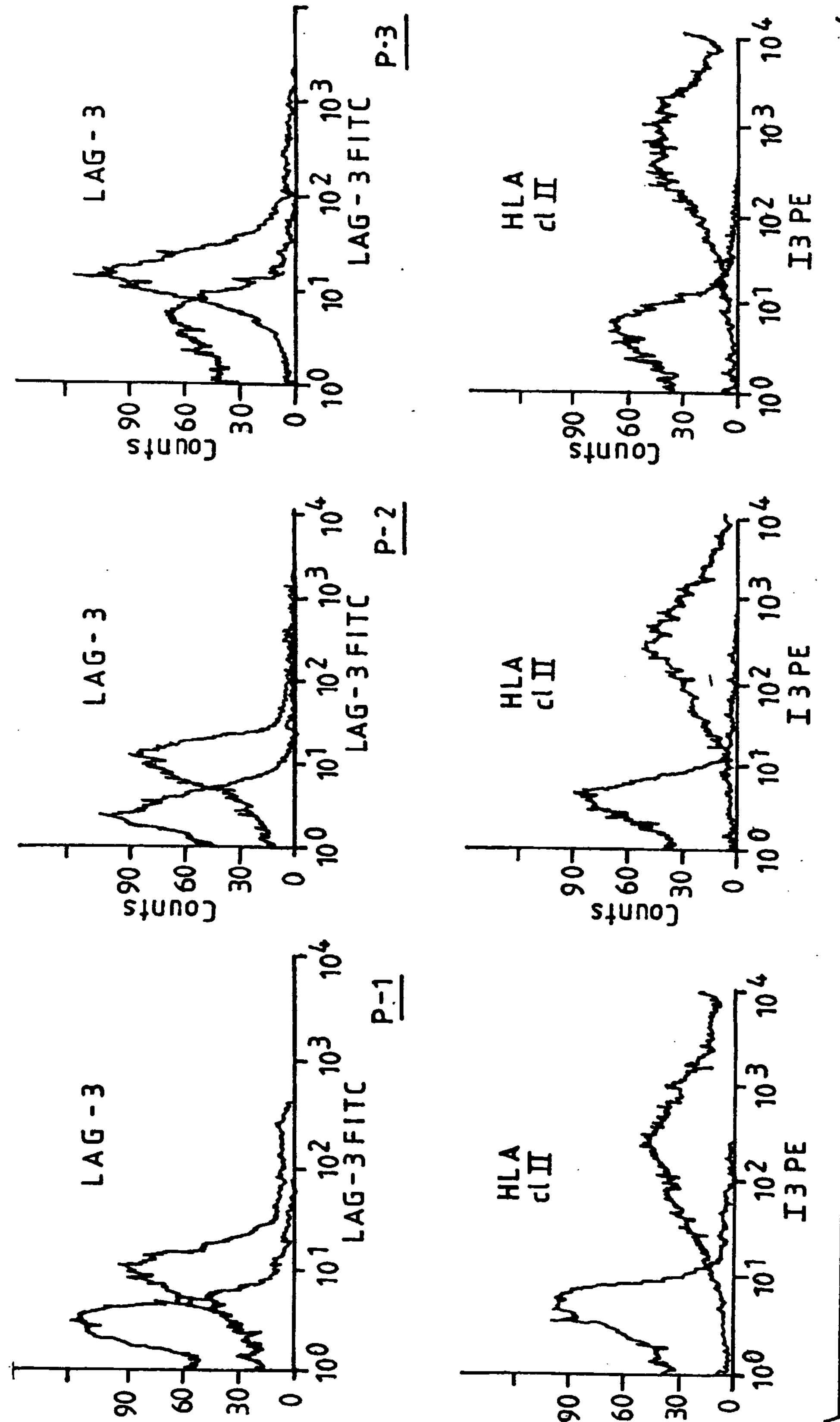

FIG.7

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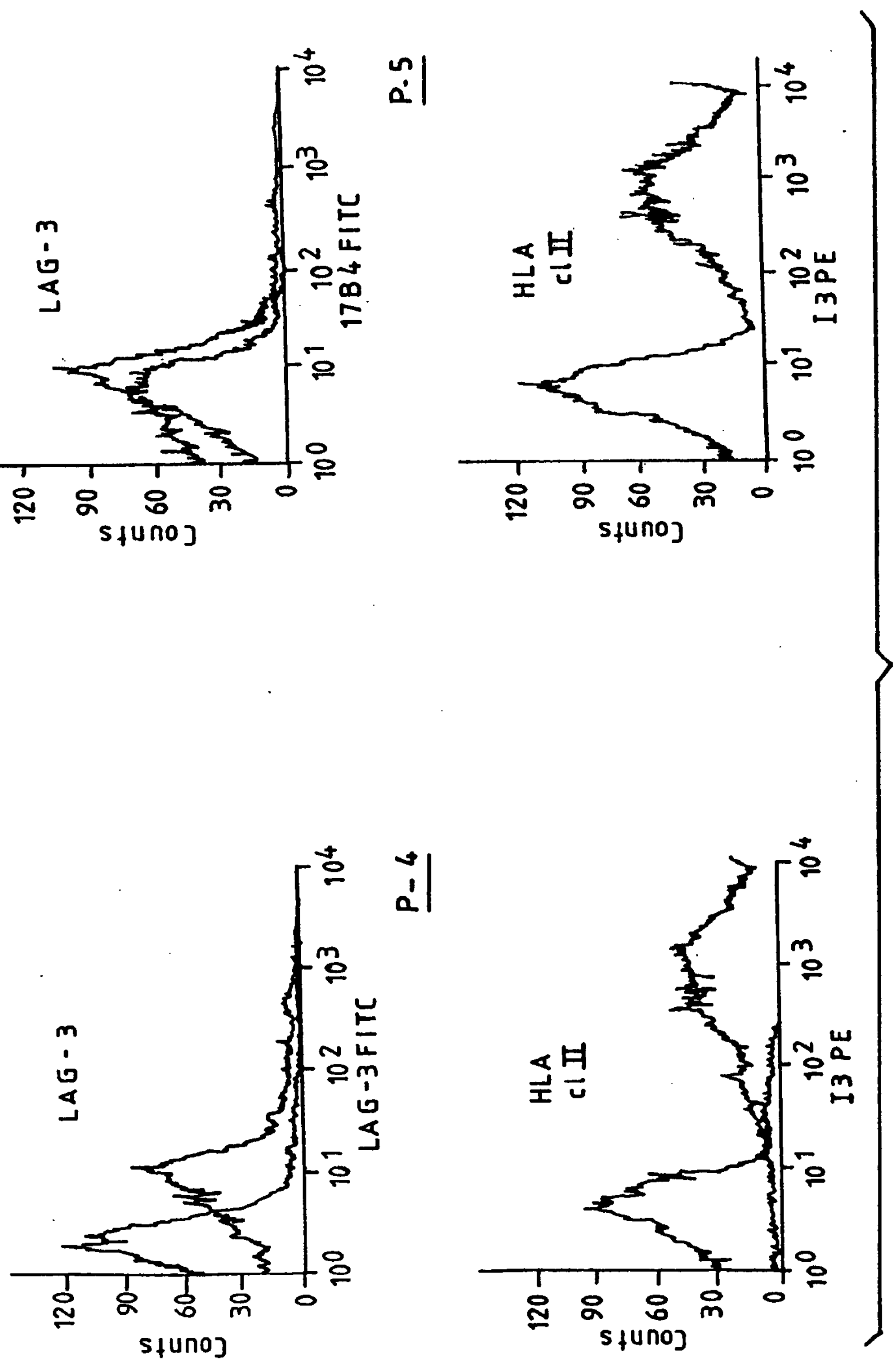

FIG. 8

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EP 97 40 1800

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X	WO 96 20215 A (LABORATOIRES OM S.A.) * abstract * * page 3, line 22-35 * * page 4, line 3-9 * * page 4, line 32 - page 5, line 34 * * page 6, line 21 - page 7, line 10; claims 1-16 * ---	1,2,7-10	C12N15/63 A61K38/17 A61K39/00 A61K48/00 C12N5/10
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Y		11-15	
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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	12 March 1998	Muller-Thomalla, K

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Y	HUARD ET AL.: "T cell MHC class II molecules downregulate T cell proliferation following LAG-3 binding" 9TH INTERNATIONAL CONGRESS OF IMMUNOLOGY: SAN FRANCISCO, CALIFORNIA, USA, XP002050654 July 23-29, 1995, page 216 * abstract no. 1282 * ---	11-15	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
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X	WO 91 10682 A (INSERM) 25 July 1991 * page 32, line 7 - page 33, line 34; claims 24-29 * --- -/--	18	

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Y	HUARD ET AL.: "Cellular expression and tissue distribution of the human LAG-3-encoded protein, an MHC class II ligand" IMMUNOGENETICS, vol. 39, 1994, page 213-217 XP002058640 * page 216, column 2, paragraph 3 * -----	16-19	
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